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THE MOBILITY AND DIFFUSION COEFFICIENT OF POTASSIUM IN GIANT AXONS FROM *SEPIA*

BY A. L. HODGKIN AND R. D. KEYNES

From the Physiological Laboratory, University of Cambridge

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The object of the experiments described here is to measure the mobility and diffusion coefficient of the potassium ions inside the giant axons of *Sepia officinalis*. The principle of the method is to make a short length of axoplasm radioactive by allowing internal potassium to exchange with externally applied ^{42}K in a restricted region. If a fibre which has been treated in this way is washed with sea water and placed in oil, most of the ^{42}K remains in the axoplasm since the extracellular space is relatively small and the external potassium concentration is low compared to the internal concentration. The movement of the radioactive potassium inside the nerve fibre under the influence of diffusion or an electric field can then be studied by moving the fibre horizontally over a suitably screened Geiger counter. In the theoretical section it is shown that the mobility can be obtained from the velocity with which the radioactive patch drifts along the fibre under the influence of an applied voltage gradient, while the diffusion coefficient can be calculated from the extent to which the patch broadens during the experiment. The voltage gradient along the axis cylinder may be measured with an external electrode since internal and external gradients should be equal near the middle of a sufficiently long interpolar region (Hodgkin & Rushton, 1946).

A preliminary account of this work was given at the XVIIIth International Physiological Congress (Hodgkin & Keynes, 1950).

The discussion contains a brief account of further experiments of the type described by Keynes (1951) and Keynes & Lewis (1951*a*). These suggest that at least 90% of the total potassium is free to exchange with radioactive potassium, and therefore provide evidence that the results of the present study apply to the bulk of the potassium inside the axoplasm.

METHOD

The only new apparatus required was an arrangement for moving the nerve fibre above the aperture of a screened Geiger counter. This underwent some modification during the experiments but we shall describe in detail only the final version (Fig. 1 A) since this design was the most satisfactory and the same general principle was used throughout. In its final form (which was used in the last three experiments), the apparatus consisted of a Geiger counter surrounded by a lead cylinder and covered with a block of brass in which there was a circular window of width 1 cm. The reason for using such a wide aperture is that a smaller one would reduce the counting rate

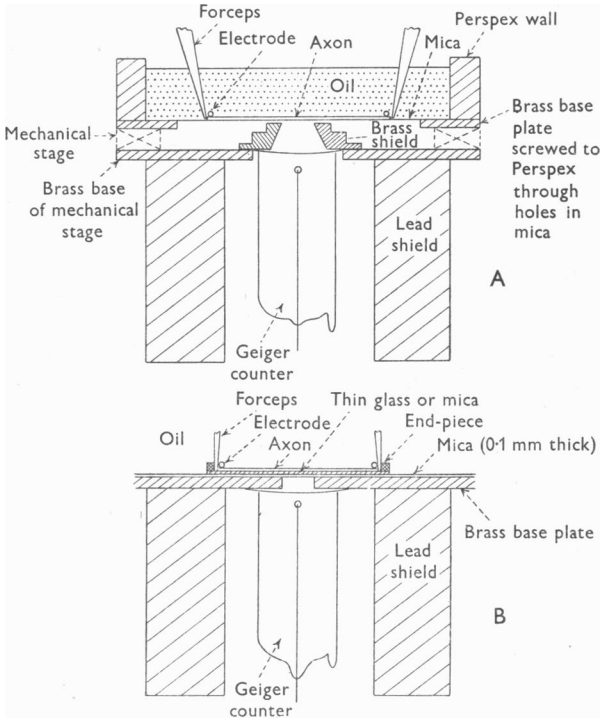

Fig. 1. A. Diagram of final version of nerve chamber and Geiger counter. Only the edges of the brass base-plate supporting the mica are seen since the section passes through the slot cut in the region occupied by the nerve. Details of the mechanical stage are not shown. B. Sketch showing arrangement used in the majority of experiments. The thickness of the mica sheet and of the glass or mica strip has been exaggerated.

from a single fibre to a point at which random errors would offset any increase in resolution. A mechanical stage with 7 cm of horizontal travel was built on to the Geiger counter assembly and carried the nerve chamber. The latter was filled with oil and consisted of an open rectangular box with thick Perspex walls and a mica bottom 0·1 mm in thickness. The mica was supported by a brass base plate out of which a large slot was cut in such a way that the window above the Geiger counter lay just below the nerve. The forceps for holding the nerve and the electrodes for applying current were attached to the Perspex walls of the cell through a vertical rack and pinion which enabled the nerve to be lifted off the bottom of the cell. The silver chloride electrodes were

mounted in glass tubes filled with agar sea water which terminated in agar wicks about 1 mm in diameter. A similar electrode with a finer wick was used to measure the voltage gradient.

The cell used in the first eight experiments is shown in Fig. 1 B. In this apparatus the oil bath was fixed and the nerve rested on a sliding carriage which was moved horizontally over the mica bottom of the oil bath. The carriage consisted of a long piece of glass or mica about 0·15 mm in thickness on to which end pieces of glass were cemented. It was held horizontally by outward pressure from the forceps holding the nerve.

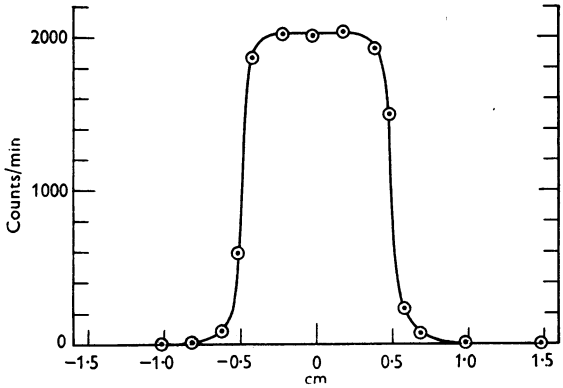

Fig. 2. Calibration of screened Geiger counter shown in Fig. 1 A. The abscissa gives the horizonta distance between the vertical axis of the Geiger counter and a small crystal of radioactive KCl placed on the mica bottom of the nerve chamber. The ordinate is the counting rate.

The diameter of the Geiger counter window was 1 cm in all the experiments. Its screening properties were examined by placing a small crystal of ^{42}KCl in the position normally occupied by the centre of the nerve. When the crystal was moved over the Geiger counter the curve shown in Fig. 2 was obtained. It will be seen that the screen allows radiation to be collected over a distance of about 1 cm and then cuts off sharply. This curve was obtained with the apparatus in its final form. The earlier version gave a curve which was similar except that the maximum was less flat.

On one occasion the apparatus was tested by observing the movement of a patch of ^{42}KCl injected into a thin-walled capillary (500 μ diameter) filled with 0·2 M-KCl. The result was within 2 % of that calculated from the conductivity of the solution, but a figure of ± 5 % is more likely to represent the accuracy of this experiment.

Artificial sea-water solutions containing ^{42}K were made up with K concentrations varying between 10 and 50 mM (usually 20 mM), and concentrations of other ions approximately as stated by Keynes (1951, table 1). Sea water or artificial sea water containing 10 mM-K were used for dissection.

Radioactive samples were prepared by irradiating spectroscopically pure K_2CO_3 or 'Analar' $KHCO_3$ in the neutron pile at A.E.R.E. Harwell. In the first case the carbonate was dissolved in a little water, and neutralized to pH 7·0 with 1 N-HCl, CO_2 being pumped off at reduced pressure and pH measured with a glass electrode. In the second, K was precipitated as $KClO_4$ and reduced to KCl by the general method described by Keynes & Lewis (1951 *a*). Artificial sea water was made up from the ^{42}KCl and applied to the nerve as a drop about 7 mm in diameter. This was held in a small Perspex cup which was removed when the centre of the axon had taken up sufficient ^{42}K.

The apparent concentrations of labelled potassium given in Table 1 were obtained by dividing the maximum counting rate obtained from the nerve by a factor obtained from a 200 μ capillary

filled with a labelled KCl solution of known concentration. A numerical analysis using a typical distribution (Fig. 3) and a calibration curve (Fig. 2) indicated that the maximum concentration at the centre of the radioactive patch was about 20% higher than the figures given in Table 1.

For the exchange experiments mentioned on p. 526 the general methods were those of Keynes (1951) and Keynes & Lewis (1951 *b*).

Giant axons 4–6 cm long and about 200 μ in diameter were dissected by the method described by Keynes (1951) and carefully cleaned from adherent tissue. It was important to use long axons since Weidmann (1951) gives the space constant of electrotonic spread as 5·7 mm and measurements of the type described here should ideally be made at a distance of 15 mm from the anode. Remoteness of the cathode is also desirable, but is less important since the membrane resistance decreases under a cathode and the effective space constant is greatly reduced (cf. Cole, 1941).

THEORETICAL SECTION

Diffusion and electrical transport

It will be assumed that the flow of ^{42}K along the axis cylinder is given by

$$m = -D\frac{\partial y}{\partial x} - uv'y, \tag{1}$$

where m is the flow of ^{42}K through unit area, y is the concentration of ^{42}K, x is distance along the nerve, D is the diffusion coefficient, u is the mobility and v' is the voltage gradient. In a dilute solution, $D=(RT/F)u$, but this substitution will not be made since D and u can be measured independently. The rate at which the concentration of ^{42}K increases with time is given by $-\partial m/\partial x$. Since v' is constant it follows that

$$\frac{\partial y}{\partial t} = D\frac{\partial^2 y}{\partial x^2} + uv'\frac{\partial y}{\partial x}. \tag{2}$$

This equation can be simplified by a method suggested by Mr A. F. Huxley. Suppose that $y=f(z, t)$, where $z=x+uv't$.

Then
$$\left(\frac{\partial y}{\partial x}\right)_t = \left(\frac{\partial f(z,t)}{\partial z}\right)_t \left(\frac{\partial z}{\partial x}\right)_t = \left(\frac{\partial f(z,t)}{\partial z}\right)_t,$$

and
$$\left(\frac{\partial^2 y}{\partial x^2}\right)_t = \left(\frac{\partial^2 f(z,t)}{\partial z^2}\right)_t,$$

$$\left(\frac{\partial y}{\partial t}\right)_x = \left(\frac{\partial f(z,t)}{\partial t}\right)_z + \left(\frac{\partial f(z,t)}{\partial z}\right)_t \left(\frac{\partial z}{\partial t}\right)_x$$

$$= \left(\frac{\partial f(z,t)}{\partial t}\right)_z + uv'\left(\frac{\partial f(z,t)}{\partial z}\right)_t.$$

On substituting these values in equation (2) we obtain

$$\left(\frac{\partial f(z,t)}{\partial t}\right)_z = D\left(\frac{\partial^2 f(z,t)}{\partial z^2}\right)_t,$$

or
$$\left(\frac{\partial y}{\partial t}\right)_z = D\left(\frac{\partial^2 y}{\partial z^2}\right)_t. \tag{3}$$

Hence any solution $y=f(z, t)$ of the ordinary diffusion equation (3) will also satisfy equation (2) if z is replaced by $x+uv't$.

In the present case the boundary conditions are $y=f_0(x)$ when $t=0$, and $y\neq\infty$ when $x=\pm\infty$. For all finite times identical boundary conditions hold in terms of z so that the solution using z is the same as the solution using x in the absence of a voltage gradient. This means that the broadening of the radioactive patch due to diffusion and the drift of the patch under the applied field will proceed independently. If the patch is symmetrical about $x=0$ at $t=0$ it will remain symmetrical about $x=-uv't$ and will have exactly the same shape as it would in the absence of an applied field. The maximum of the patch will therefore move along the nerve in the direction of x with a constant velocity of $-uv'$. Measurements of u by this method will not be disturbed by the wide aperture of the Geiger counter since this cannot alter the position of maximum radioactivity.

To measure the diffusion coefficient we make use of the following argument. The observed radioactivity θ is related to the actual radioactivity by the relation

$$\theta=\int_{-\infty}^{\infty}\pi r^2 y\phi(\lambda)\,d\lambda, \tag{4}$$

where r is the radius of the fibre and λ is the distance measured from the centre of the Geiger counter window. $\phi(\lambda)$ is a calibration curve of the type shown in Fig. 2. Similar expressions hold for the partial derivatives of θ and y with respect to t and x. Thus

$$\frac{\partial\theta}{\partial t}=\int_{-\infty}^{\infty}\pi r^2\frac{\partial y}{\partial t}\phi(\lambda)\,d\lambda \quad\text{and}\quad \frac{\partial^n\theta}{\partial x^n}=\int_{-\infty}^{\infty}\pi r^2\frac{\partial^n y}{\partial x^n}\phi(\lambda)\,d\lambda.$$

Hence, $$-\frac{\partial\theta}{\partial t}+D\frac{\partial^2\theta}{\partial x^2}+uv'\frac{\partial\theta}{\partial x}=\int_{-\infty}^{\infty}\left(-\frac{\partial y}{\partial t}+D\frac{\partial^2 y}{\partial x^2}+uv'\frac{\partial y}{\partial x}\right)\pi r^2\phi(\lambda)\,d\lambda.$$

On substituting from equation (2) the integral vanishes and

$$\frac{\partial\theta}{\partial t}=D\frac{\partial^2\theta}{\partial x^2}+uv'\frac{\partial\theta}{\partial x}. \tag{5}$$

Thus although the observed radioactivity (θ) has a different distribution from the actual radioactivity in the nerve (y) it still obeys the same differential equation. This is a convenient result because it allows the diffusion coefficient to be measured without going through the tedious and uncertain process of converting θ into y by means of the calibration curve $\phi(\lambda)$. The method was still further simplified by the finding that the initial distribution $\theta_{x,0}=f_0(x)$ was well fitted by a Gaussian curve. The boundary condition for equation (5) is then

$$\theta_{x,0}=A_0\exp-\left(\frac{x-\bar{x}_0}{\alpha_0}\right)^2, \tag{6}$$

where A_0, $\bar{x}_0$ and α_0 are constants. With this initial condition the distribution at any subsequent time t is

$$\theta_{x,t}=A_t \exp -\left(\frac{x-\bar{x}_t}{\alpha_t}\right)^2, \tag{7}$$

where

$$\frac{A_t}{A_0}=\left(1+\frac{4Dt}{{a_0}^2}\right)^{-\frac{1}{2}}, \tag{8}$$

$$\alpha^2{}_t-{\alpha_0}^2=4Dt, \tag{9}$$

$$\bar{x}_t-\bar{x}_0=-uv't. \tag{10}$$

These are the relations used to calculate D and u.

Method of fitting curves to experimental data

The problem considered here is that of fitting equation (7) to data such as those shown in Fig. 3. The method which we finally employed was suggested by Mr Huxley; it consisted in expressing the experimental data θ_1, x_1, θ_2, x_2, etc., in the form w_1, q_1, x_1, w_2, q_2, x_2, where w is the weight attached to each observation and $q=\ln \theta$.

These values must now be fitted by a parabola of the form $a+bx+cx^2$, where $a=\ln A-\bar{x}^2/\alpha^2$, $b=2\bar{x}/\alpha^2$, $c=-1/\alpha^2$. Since each observation of weight w is equivalent to w observations of equal weight it follows that the data are best fitted when the quantity $\Sigma w(a+bx+cx^2-q)^2$ is a minimum. On differentiating this expression with respect to a, b and c and equating to zero the following relations are obtained:

$$\Sigma wq=a\Sigma w+b\Sigma wx+c\Sigma wx^2, \tag{11}$$

$$\Sigma wqx=a\Sigma wx+b\Sigma wx^2+c\Sigma wx^3, \tag{12}$$

$$\Sigma wqx^2=a\Sigma wx^2+b\Sigma wx^3+c\Sigma wx^4. \tag{13}$$

The weight attached to each observation is obtained in the following manner. Provided that the counting rate is not close to background the best estimate of the variance of an observation θ is proportional to the total number of counts (n) recorded by the counter. θ is related to n by

$$\theta=[\exp{(kt)}]\left[\frac{n}{\Delta t}-B\right],$$

where k is the decay constant of ^{42}K, t is time, Δt is the counting time and B is the background. Since a small error Δn is associated with an error of $(\partial q/\partial n)\Delta n$ in q, it follows that the variance of q is approximately:

$$V(q)=V(n)\left(\frac{\partial q}{\partial n}\right)^2=\frac{n}{(n-B\Delta t)^2}.$$

The weight to be attached to any reading is therefore $(n-B\Delta t)^2/n$. Observations in which the counting rate was not significantly different from background were rejected in order to avoid the difficulties arising from occasional negative values of θ.

Having found values for a, b, c by means of equations (11), (12) and (13), it was then a simple matter to obtain A, α and $\bar{x}$ by the relations

$$A=\exp(a-b^2/4c), \qquad \alpha=\sqrt{(-1/c)} \quad \text{and} \quad \bar{x}=-b/2c.$$

RESULTS

After the axon had been isolated and cleaned it was mounted in oil about 1 cm above the bottom of the cell shown in Fig. 1. The centre of the axon was soaked for 1–3 hr in a large drop of artificial sea water containing 10–50 mM of K labelled with ^{42}K. Under these conditions labelled potassium (K*) exchanged with the internal potassium over a length of 1 cm and reached an apparent concentration of 10–100 mM, the amount being roughly proportional to the duration of treatment and the potassium concentration in the external solution. At the end of this period the drop of ^{42}K was removed and external ^{42}K washed off with sea water. The excitability was then tested over the whole length of the axon. Shortly afterwards the voltage gradient produced by a longitudinal current of about 3·5 μA was measured by means of a fine wick electrode. The current was applied between electrodes about 5 cm apart and the voltage gradient it produced was recorded over the central stretch of axon. In this region there should be little membrane current since the voltage gradient was usually recorded at a distance of several space constants from the electrodes. It is therefore reasonable to take the external voltage gradient as equal to the internal gradient along the axis cylinder. A similar measurement, made at the end of the experiment with the same current, was found to agree with the first to within a few per cent. Most of the axons tapered slightly and the voltage gradient often increased by 5–10% per cm as the recording electrode was moved away from the central end of the fibre. In order to reduce errors from this cause the voltage gradient used for calculating the mobility was taken as the mean over a length of 2 cm in the region occupied by ^{42}K (e.g. from 1·5 to 3·5 cm in Fig. 3). When this measurement was complete the current was switched off and the axon was lowered on to the mica bottom of the cell (Fig. 1 A) or on to a thin strip of mica or glass (Fig. 1 B). The distribution of ^{42}K was determined by sliding the fibre over the Geiger counter window and taking counts at a number of fixed positions. In most of the experiments an attempt was made to 'bracket' the maximum by making alternate measurements on either side in quick succession. The whole operation took 20–30 min and resulted in a series of points such as those shown in Fig. 3 A or Fig. 4 A. The next stage was to switch on the current for a period

of 40–80 min. During this period the counting rate was recorded at certain fixed distances. This is illustrated by Fig. 5 which shows the counting rate, determined in 1 min counts at 1·5, 2·7 and 3·1 cm. At 1·5 cm the counting rate decreased steadily whereas it increased at 2·7 cm, indicating that the

TABLE 1

Axon	Temp. (° C)	Voltage gradient (V/cm)	Velocity (mm/min)	Mobility (10^{-4} cm^2 sec^{-1} V^{-1})	Diffusion coefficient (from α) (10^{-5} cm^2/sec)	Diffusion coefficient (from A) (10^{-5} cm^2/sec)
1	18	−0·400	0·138	5·75	0·73	2·28
2	18	−0·432	0·125	4·82	—	—
3	19	−0·331	0·091	4·60	—	—
4	17	−0·548	0·177	5·37	2·13	1·53
5	16	−0·295	0·096	5·42	1·12	1·16
6	19	−0·600	0·185	5·14	2·24	2·99
7	18	+0·415	−0·095	3·80	0·52	0·79
8	18	0	(0·005)	—	1·09	1·88
9	19	−0·287	0·076	4·39	1·65	1·69
10	17	0	(0·001)	—	1·06	0·89
11	17	+0·412	−0·113	4·57	1·13	2·34
Mean	18	—	—	4·87	1·30	1·73
S.E. of mean	—	—	—	0·20	0·20	0·24

Experimental details

Axon	Diameter (μ)	Time soaked in drop (min)	Concentration K* (mM) in drop	Concentration K* (mM) in nerve	Current (μA)	Duration of current (min)	Length excitable (mm) Beginning	Length excitable (mm) End
1	210	125	50	86	5·43	80	40	0
2	134	145	10	18	2·26	65	28	10
3	218	160	20	35	3·98	83	33	20
4	181	65	20	11	3·90	37	24	10
5	185	65	20	10	2·33	62	30	25
6	181	150	20	32	4·72	46	25	0
7	183	65	20	7	−3·92	80	42	36
8	151	130	20	20	0	(105)	30	20
9	218	75	20	10	3·69	85	40	23
10	200	215	20	35	0	(445)	20	0
11	164	136	20	37	−2·91	63	20	0

Voltage gradients, velocities and currents are given in the peripheral direction (i.e. from the head end to the tail end of the fibre). The figures for the concentrations of labelled potassium (K*) in the nerve are approximately equal to the average concentrations of K* over 1 cm in the middle of the radioactive patch. The maximum concentration at the centre of the patch was probably about 20% greater. The counting rates in axons 2 and 3 were too low to determine diffusion coefficients. The bracketed 'durations' in axons 9 and 11 give the interval between the initial and final determinations of the distribution of ^{42}K. The corresponding figure in the other experiments was about 25 min greater than the duration of current.

maximum was moving in the direction of $+x$. The two counting rates became equal 11 min after the current was switched on, showing that the maximum was located at 2·1 cm at this time. The line for 3·1 cm intersects that for 1·5 cm at 23 min indicating that the maximum moved 2 mm in 12 min. The straight

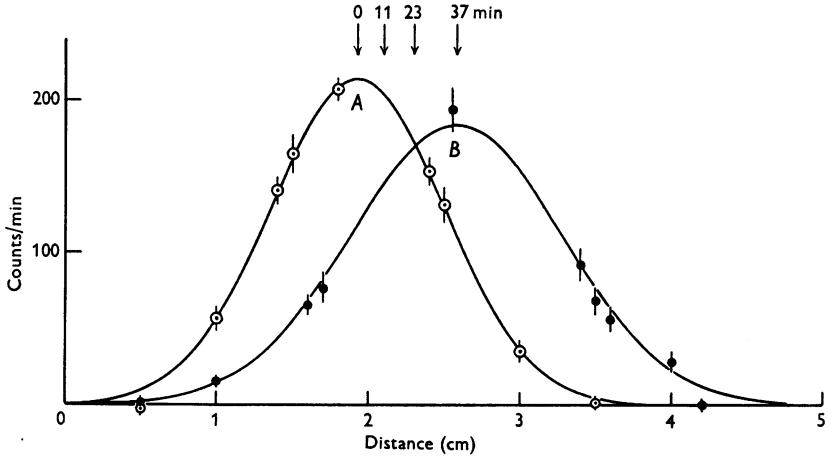

Fig. 3. Distribution of radioactivity along nerve. Curve A (⊙—⊙) gives the observed radioactivity (ordinate) as a function of distance along the nerve at the beginning of the experiment. Curve B (●—●) is a similar distribution at the end of the experiment. Between making curves A and B a current producing a voltage gradient of $-0{\cdot}548$ V/cm was applied for 37 min. The arrows show the position of the maximum at various times. The anode was located at the central end of the fibre ($-0{\cdot}05$ cm) and the cathode at the peripheral end (4·2 cm). Other experimental details given under axon 4, Table 1. The vertical lines drawn through the points show ±one S.D. The smooth curves are drawn from equations (6) or (7) with the parameters given in the text.

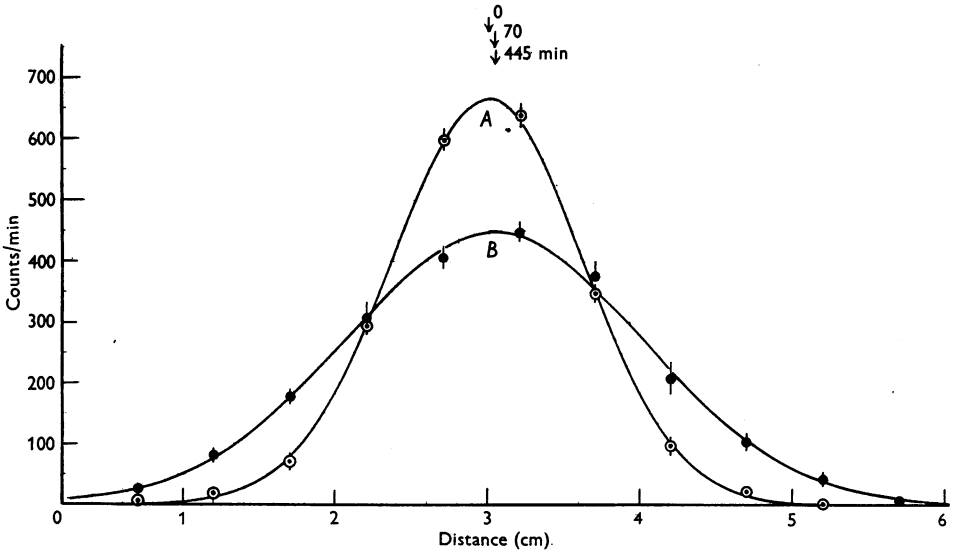

Fig. 4. Control experiment with no voltage gradient (axon 10, Table 1). Curve A shows the initial distribution of radioactivity and curve B the final distribution 445 min later. The arrows show the position of the maximum at various times. Vertical lines give ±S.D. The smooth curves are drawn according to equations (6) or (7) with $A_0 = 666$ counts/min, $A_t = 447$ counts/min, $\alpha_0 = 0{\cdot}883$ cm, $\alpha_t = 1{\cdot}384$ cm, $\bar{x}_0 = 2{\cdot}991$ cm and $\bar{x}_t = 3{\cdot}035$ cm. The central end of the fibre was at 0·2 cm and the peripheral end at 6·2 cm.

lines shown in the figure were calculated by the method of least squares using a formula appropriate to a situation in which the variance $V(\theta)$ of any observation θ is equal to θ. This method was used in about half the experiments but was not applied in every case since it gave results which were very close to those obtained by drawing straight lines by eye.

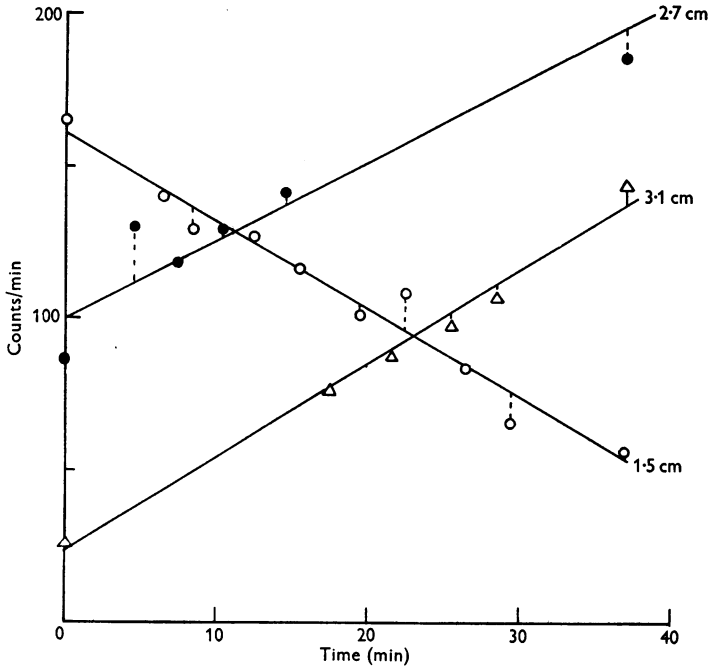

Fig. 5. Variation of counting rate with time during period of current flow at three positions on nerve (axon 4). The points at zero time and at 37 min were derived from the initial and final distribution curves. The standard deviation of the remaining points is approximately equal to the square root of the ordinate.

After 40–80 min the current was switched off and the distribution of radioactivity again determined. It will be seen from Fig. 3 that the patch of ^{42}K has broadened and that its maximum has shifted towards the cathode. In similar experiments in which no current was applied, the patch broadened as before but the maximum did not move along the nerve. This is illustrated by Fig. 4 in which the axon was left at rest for 445 min.

When the final distribution had been determined the axon was lifted from the bottom of the cell in order to check the voltage gradient and measure the excitability. In all cases in which current was applied it was found that 1 or 2 cm in the region of the electrodes were totally inexcitable. This is not surprising because the currents used were of the order of ten times threshold and lasted for about 1 hr. However, in six cases out of nine the axon gave

a propagated action potential of normal amplitude over a length of 1·0–3·6 cm in the region in which the mobility had been determined. While this result is not as satisfactory as one might wish, it is hardly surprising in view of the large currents used. The survival of the central region in six axons is presumably due to the virtual absence of membrane current in the interpolar stretch of a uniform fibre. The failure in three axons could be attributed to discontinuities in the amount of external fluid but might equally have nothing to do with the current since some failures are inevitable in long experiments of this type. There is, in any case, no evidence to suggest that loss of excitability caused any large change in mobility. Inspection of Table 1 shows that the mobility in the three fibres which became inexcitable before the end of the experiment did not differ significantly from those in the remaining fibres. Nor was there any systematic tendency for the mobility to alter during the period of current flow, as may be seen from points such as those shown in Fig. 6.

The experiment which has been described allows both the mobility and the diffusion coefficient to be estimated. The analysis consisted in fitting Gaussian curves by the method described on p. 518. The resulting curves are clearly a good fit to the experimental points as may be seen from the typical result in Fig. 3B. (The fit in Fig. 3A is better than that usually obtained.) The numerical process of obtaining two Gaussian curves gave six quantities which had the following values in the experiment illustrated by Fig. 3:

A_0 (initial amplitude) $= 214$ counts/min
A_t (final amplitude) $= 184$ counts/min
α_0 (initial 'width') $= 0{\cdot}810$ cm
α_t (final 'width') $= 0{\cdot}989$ cm
$\bar{x}_0$ (initial position of maximum) $= 1{\cdot}928$ cm
$\bar{x}_t$ (final position of maximum) $= 2{\cdot}581$ cm.

The duration of the current in this experiment was 37 min and the mean voltage gradient was $-0{\cdot}548$ V/cm. Hence $u = 5{\cdot}37 \times 10^{-4}$ cm^2 sec^{-1} V^{-1} (from equation 10).

The interval between the mid times of curves A and B was 63 min and this time rather than the duration of current must be used to calculate D. Using the difference $\alpha_t^2 - \alpha_0^2$, D is found to be $2{\cdot}13 \times 10^{-5}$ cm^2/sec (from equation 9) while a value of $1{\cdot}53 \times 10^{-4}$ cm^2/sec is obtained from A_0, A_t and α_0 (equation 8). Of the two methods the former is thought to be more reliable, but neither is at all accurate since a small change in α or A produces a large error in D.

The method of calculating the mobility described in the previous paragraph was not used in Table 1 since it took no account of the determinations made during the period of current flow. The two sets of measurements are combined

in Fig. 6 which shows that the point of maximum radioactivity moved along the fibre at constant speed. The straight lines in these figures are the regressions of x on t and a similar method was adopted in calculating all the velocities shown in Table 1. Mobilities were then obtained by dividing the velocity by the voltage gradient.

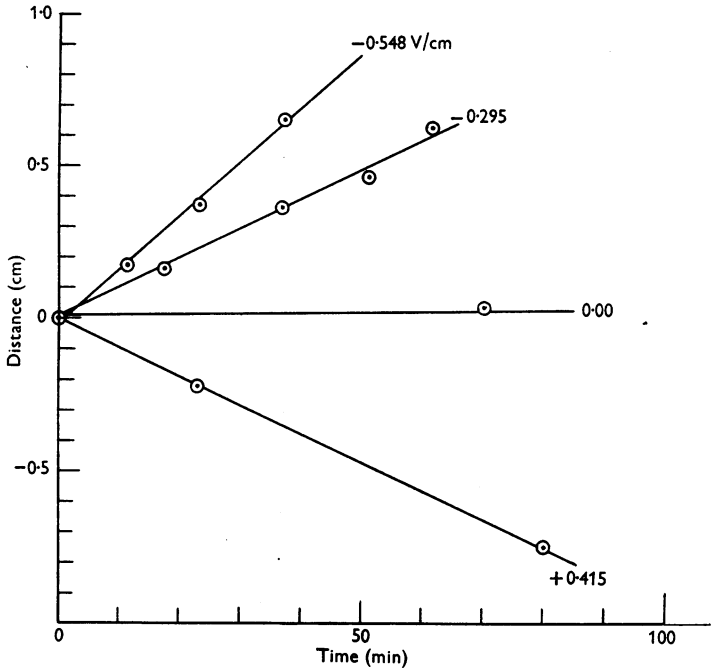

Fig. 6. Variation of position of maximum radioactivity with time. The ordinate gives the position of the maximum relative to its position at the beginning of the experiment; a movement from the central to the peripheral end of the fibre is taken as positive. The figures attached to each curve show the voltage gradient; a negative sign means that the cathode is at the peripheral end of the fibre. The straight lines are the regression of distance on time. A point at 445 min was included in calculating the line at zero voltage. Experimental data taken from axons 4, 5, 10 and 7. In each case initial and final points were obtained by the method illustrated in Fig. 3 while intermediate points were obtained by the method illustrated in Fig. 5.

The main sources of inaccuracy in the determination of the mobility are probably those introduced by counting errors and by changes in axon diameter. In the early experiments there may have been a small amount of backlash between the moving stage and the slide on which the fibre rested (Fig. 1 B). This probably did not amount to more than about 0·2 mm and was completely eliminated in the last three experiments in which the arrangement shown in Fig. 1 A was employed. In this case the backlash was less than the accuracy of setting the mechanical stage which was of the order of 0·03 mm.

It is difficult to estimate the extent to which any of these effects might have introduced systematic errors but it seems unlikely that the mean mobility could have been much lower than 4×10^{-4} cm^2 sec^{-1} V^{-1} or much higher than $5{\cdot}5 \times 10^{-4}$ cm^2 sec^{-1} V^{-1}.

One objection which might be raised is that potassium ions may be moving in the external fluid rather than in the axoplasm itself. This effect might be serious if the mobility of sodium or chloride ions were measured by the same method but it is thought to be very small in the present experiments. According to Weidmann (1951) and Keynes & Lewis (1951*b*) a *Sepia* axon immersed in oil is surrounded by about 10 μ of sea water. This means that the volume of external fluid is about one-fifth of that of the axoplasm. Leakage might raise the potassium concentration in the external fluid but it could hardly make it greater than one-fifth of that in the axoplasm. The total quantity of ^{42}K in the external fluid should therefore have been less than 4% of that in the axis cylinder. This conclusion was confirmed by the observation that not more than about 5% of the total radioactivity of the nerve and external fluid could be removed by washing with sea water for a few minutes. This amount of ^{42}K would only have to be taken into account if the observed mobility were very much less than that in the external fluid. In fact the average mobility was about 90% of that in sea water (see p. 520) so that movement in the external fluid may safely be ignored. Qualitative evidence for this conclusion was obtained in a preliminary experiment which showed that ^{42}K moved towards the cathode in a fibre which was washed intermittently by wiping a drop of sea water along the fibre in the opposite direction.

DISCUSSION

The experiments described here allow the behaviour of potassium ions inside a nerve fibre to be compared with that in free solution. The first point to be examined is the relation between the mobility (u) and the diffusion coefficient (D). In a dilute solution (Nernst, 1923; Glasstone, Laidler & Eyring, 1941) the diffusion coefficient should be given by

$$D = (RT/Z_i F)\, u,$$

where Z_i is the valency of the ion, and R, T and F have their usual significance. If potassium exists as a free ion Z_i should be $+1$ so that $D = u \times 0{\cdot}025$ V. The value of D found by the first and possibly more reliable method is $1{\cdot}30 \times 10^{-5}$ cm^2/sec (S.E. $= 0{\cdot}20$) while that found by the second is $1{\cdot}73 \times 10^{-5}$ cm^2/sec (S.E. $= 0{\cdot}24$). Neither value is significantly different from the theoretical value of $1{\cdot}22 \times 10^{-5}$ cm^2/sec (S.E. $= 0{\cdot}05$) predicted from the observed mean value of u ($4{\cdot}87 \times 10^{-4}$ cm^2 sec^{-1} V^{-1}, S.E. $= 0{\cdot}2$).

The values of u and D for axoplasm are evidently close to those in an aqueous solution of the same concentration. The equivalent conductivity of

0·5 M-KCl is 102·41 mho cm^2 mole^{-1} at 18° C (Landolt-Börnstein, 1923) and the potassium transference number is 0·49 (MacInnes, 1939). The mobility is therefore $0{\cdot}49 \times 102{\cdot}41/96{,}500 = 5{\cdot}2 \times 10^{-4}$ cm^2 sec^{-1} V^{-1}. We do not know of any accurate measurements of the self diffusion coefficient of K in KCl but it should be fairly close to the diffusion coefficient of KCl which is $1{\cdot}5 \times 10^{-5}$ cm^2/sec in an 0·5 M solution at 18° C (Landolt-Börnstein, 1931). Our measurements therefore suggest that the ^{42}K which enters an axon exists in the axoplasm in much the same state as in an 0·5 M-KCl solution. This does not prove that all the potassium inside the nerve consists of free ions since an appreciable fraction of the potassium might be bound so firmly that it is unable to exchange with the radioactive potassium which enters the fibre. A suggestion of this kind has been made for the squid axon by Rothenberg (1950), but his argument is open to the objection that constancy of total internal potassium in fibres soaked for different periods was assumed but not established by chemical analysis (see Keynes & Lewis, 1951*a*). In an earlier paper (Keynes, 1951) an experiment was described which suggested that at least 85 % of the potassium in *Sepia* axoplasm was free to exchange with ^{42}K in the external solution. This experiment has now been repeated a number of times in a slightly modified form. As before, axons were first soaked in radioactive sea water for a period of about 3 hr, short counts being taken at hourly intervals to measure the rate of gain of ^{42}K. When an appreciable proportion of the potassium had been exchanged, the axons were transferred to inactive sea water, and counted for a further hour to measure the rate of loss of ^{42}K. The modifications consisted in using solutions containing extra potassium, so as to increase the exchange rate, and in determining the actual potassium content of each axon at the end of the experiment by activation analysis (Keynes & Lewis, 1951*b*). In nine experiments where the potassium concentration was five times normal (51·5 mM), done at a mean temperature of 18° C, an average of 55 % of the total intracellular potassium was exchanged in the first 3 hr. Only part of the potassium in the axoplasm could have exchanged in this limited time, but from the ratio of rate of gain to rate of loss of ^{42}K at the moment of removal to inactive sea water, and the ratio of potassium influx to potassium outflux, both of which were measured fairly reliably in the course of the experiment, it was possible to calculate the level towards which exchange was proceeding (Keynes, 1951, equation 3). It was thus found that the average content of exchangeable potassium corresponded to 100 ± 6 % (S.E. of mean) of the total potassium present. Three similar experiments at 13° C in which the external potassium concentration was 20·6 mM gave an exchange of 14 % in the first 3 hr, and a calculated free potassium content of 80 ± 13 %.

Although these experiments still do not rule out the possibility that a small fraction of the internal potassium might in some way be tightly bound, they provide satisfactory confirmation that the proportion of potassium in the

axoplasm which is not free to exchange cannot be higher than about 10% of the total. Since the potassium which is free to exchange has nearly the same mobility as in free solution it is legitimate to conclude that the bulk of potassium inside an axon exists as free ions.

The data in Table 1 allow an estimate to be made of the contribution of potassium ions to the total conductance of the axoplasm. According to Weidmann (1951) the ratio of external to internal resistance is about 1·9 in cleaned *Sepia* axons immersed in oil. If this ratio is assumed, the resistivity of axoplasm can be calculated from the figures for voltage gradient, current and axon diameter. The average value obtained in this way was 46 Ω cm at a temperature of 18° C. Weidmann obtained 63 Ω cm at 11–17° C but points out that this value may have been too large because the interpolar distance was not infinite as assumed in the analysis. We shall therefore take 46 Ω cm as a basis for calculation. The potassium concentration of the fibres in Table 1 was probably about 300 m.mole/l. axoplasm (Keynes & Lewis, 1951*b*) so that the conductance due to potassium ions may reasonably be taken as

$$300 \times 10^{-6} \times 4{\cdot}87 \times 10^{-4} \times 96{,}500 \text{ mho/cm} = 0{\cdot}0141 \text{ m.mho/cm} = (71\,\Omega\,\text{cm})^{-1}.$$

The calculation indicates that potassium ions account for 60–70% of the axoplasm conductivity, or—to be more precise—that the transport number of potassium in axoplasm is 0·6–0·7.

SUMMARY

1. The mobility and diffusion coefficient of potassium ions in axoplasm were measured by studying the movements of ^{42}K which had been allowed to accumulate in a short length of a giant axon from *Sepia officinalis*.

2. The distribution of ^{42}K at various times agreed with equations based on the assumption that these ions are free to move under the influences of diffusion and the electric field.

3. At 18° C the average mobility was $4{\cdot}9 \times 10^{-4}$ cm^2 sec^{-1} V^{-1}, while the average diffusion coefficient was about $1{\cdot}5 \times 10^{-5}$ cm^2 sec^{-1}. These values are close to those for an 0·5 M-KCl solution.

4. A separate set of experiments in which axons were soaked in solutions containing ^{42}K and subsequently analysed indicated that at least 90% of the potassium was free to exchange.

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